SURVIVAL AND GROWTH OF JUVENILE LOST RIVER SUCKERS (*DELTISTES LUXATUS*) CHALLENGED WITH A BACTERIAL PATHOGEN (*FLAVOBACTERIUM COLUMNARIS*) DURING EXPOSURE TO SUBLETHAL AMMONIA CONCENTRATIONS AT pH 9.5

Final Report
Submitted to:

Dr. Elaine Snyder-Conn
U.S. Fish and Wildlife Service
Klamath Falls Fish and Wildlife Office
6610 Washburn Way
Klamath Falls, OR 97603

Submitted by :

Michael J. Suedkamp
Jeffrey M. Morris
Hilary M. Lease
Susan J. Clearwater
Joseph S. Meyer
Department of Zoology and Physiology
University of Wyoming
Laramie, WY 82071-3166

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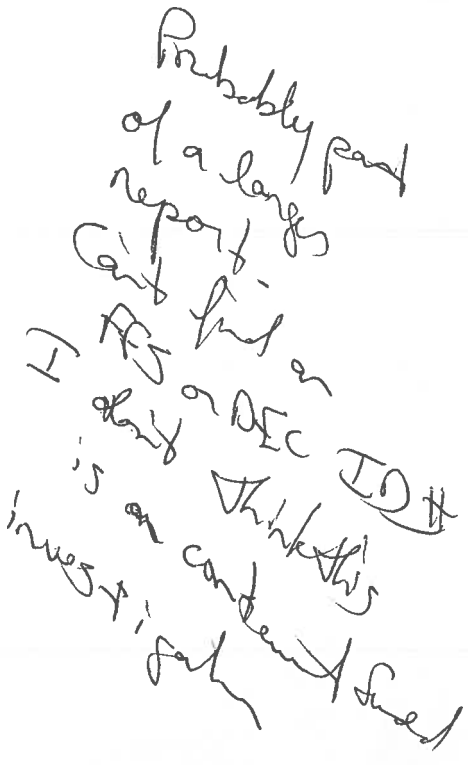

INTRODUCTION

From 30 September to 1 December 1999, we tested survival and growth of Lost River suckers (*Deltistes luxatus*) that were exposed for 62 days to sublethal ammonia concentrations at pH 9.5 and were challenged halfway through the test with a bacterial pathogen (*Flavobacterium columnaris*). Methods used and data collected for water chemistry, survival and growth are presented in this report.

MATERIALS AND METHODS

Test organisms

Five-month-old larval Lost River suckers were shipped to the University of Wyoming from the Klamath Tribes Native Fish Hatchery (KTNFH) in Chiloquin, Oregon. They arrived at Red Buttes Environmental Biology Laboratory (RBEBL; 15 km south of Laramie, Wyoming) on 8 September 1999, three weeks prior to the initiation of the experiment. Fish in the receiving bags were aerated immediately and inspected by a biologist from the Wyoming Department of Game and Fish prior to being transferred to two holding tanks. We gradually acclimated the fish to laboratory water at 22 C, pH 7.9 and hardness of 110 mg/L as $CaCO_3$ and held them under these conditions until 15 September, when we slowly adjusted the water to 21.5 C, pH 9.5 and hardness of 50 mg/L as $CaCO_3$.

Feeding

Three times daily, fish were fed refrigerated Argent Cyclops-EZE, a freeze-dried copepod product, at a rate of 2.6% of body weight/feeding/day. Additionally, the fish were fed laboratory-reared, frozen Argentemia™ *Artemia* nauplii once per day, at 3% of their body weight. The fish were fed the same diet of Cyclops-EZE and *Artemia* nauplii at KTNFH before they were shipped to RBEBL. We periodically adjusted the feeding to compensate for growth and mortality. Visible food debris, feces and hard-water deposits were siphoned from the aquaria. After the bacterial challenge, separate pipette tips and food containers were used to feed the infected and uninfected aquaria.

Growth

On 30 September, we measured 26 fish from the stock population to determine initial lengths and weights. The total length of each fish was measured on a small fish board to the nearest mm; then, the fish was gently blotted dry, placed in a tared weighing boat, and weighed to the nearest 0.01 g. On 18 and 27 October, we netted 5 fish from each experimental aquarium (one aquarium at a time) and placed them in a 1-L beaker containing water from their aquarium. The fish were measured individually for length and weight before being returned to their aquarium. To prevent bacterial cross-contamination and/or wounds to the fish, which might have encouraged bacterial infection, we did not measure lengths and weights between the bacterial challenge (30 October) and the end of the experiment. On 1 December, all surviving fish were euthanized using buffered MS-222, one tank at a time, and then measured and weighed.

Exposure aquaria and water chemistry

Twenty-four, 8-L aquaria were fitted with individual siphons and Plexiglas lids to prevent

cross-contamination by bacteria during the test. Aquaria were arranged on the holding table using a randomization procedure based on differing ammonia concentrations and bacterial exposure. Replacement water was delivered from the diluter to each aquarium at 0.5 L/min, equivalent to ~90 volume replacements per day. On day 1, the temperature was increased 0.5°C and thereafter maintained at 22-23 C until the sixth day after the bacterial challenge (36 days after the start of the experiment), when the temperature was gradually increased to 24 C.

A light/dark cycle of 16/8 hours was maintained throughout the study. However, red lights were used at night to recover and examine dead and moribund fish.

Exposure water was prepared from a mixture of RBEBL well water and reverse-osmosis/deionized (RO/DI) water, producing a hardness of ~50 mg/L as $CaCO_3$ and an alkalinity of ~65 mg/L as $CaCO_3$. We controlled hardness by adjusting the ratio of RO/DI water; we controlled the pH at 9.5 by adding KOH or H_2SO_4, as needed.

One of four ammonia concentrations was maintained in each of 6 replicate aquaria per concentration using a proportional, flow-through diluter. The stock solution contained 1,071 mg N/L from NH_4Cl. Target concentrations were: 0.5, 0.25, 0.125, and <0.01 mg NH_3-N/L (i.e., unionized ammonia nitrogen) [or 0.84, 0.42, 0.21, and 0.01 mg N/L total ammonia nitrogen]. These concentrations did not cause significant mortality during a previous 30-d chronic toxicity test with larval Lost River suckers (Meyer et al. 2000). The ammonia exposure component of the experiment conformed to the testing guidelines of Horning and Weber (1985), ASTM (1993), and Lewis et al. (1994).

Water temperature, pH, conductivity, dissolved oxygen, and total ammonia were monitored in eight aquaria daily in rotation; thus, each aquarium was monitored once every 3 days. In addition, total hardness and alkalinity were measured in two aquaria per day. The pH was determined using an Orion Model 290 pH meter after three-point calibration of the electrode in standard buffers (pH 4, 7, and 10). Total hardness was determined using a colorimetric EDTA titration, and alkalinity was determined by titration with H_2SO_4 to pH 4.5 (APHA et al. 1995). Conductivity was determined with a VWR Scientific conductivity probe calibrated daily using VWR 1000-µmol conductivity standard. A YSI Model 58 dissolved oxygen meter was used to determine dissolved oxygen (D.O.) concentration. The D.O. meter was calibrated daily in water from a separate 40-L aquarium that was analyzed for D.O. concentration by Winkler titration. Total ammonia concentrations were measured using a colorimetric assay (Verdouw et al. 1978).

We calculated unionized ammonia concentration from temperature, pH, and total ammonia concentration using equations in Emerson et al. (1975).

Test protocol

At the start of the experiment, we transferred juvenile Lost River suckers from the stock population into buckets and randomly netted them, two at a time, into each of the 24 experimental aquaria and a separate 1-L beaker, until a total of 25 fish were placed in each aquarium and 26 fish were placed in the beaker. We measured and weighed the fish in the beaker and returned them to the stock population.

Fish were maintained under the water-quality conditions, ammonia concentrations and feeding protocol described above for 30 days prior to the bacterial challenge, and then for 6 days following the bacterial challenge. On day 36, the temperature was raised to 24 C for the remainder of the test to determine if mortality would increase at the higher temperature. All of the other water-quality parameters remained constant during the entire experiment.

Contamination precautions

Several strategies to prevent bacterial contamination of immediate and surrounding laboratory areas were initiated immediately before the bacterial challenge. All personnel assisting with the test wore rubber boots, aprons and gloves during the bacterial challenge and the remaining 32 days of the test. The lab clothing was exchanged with street clothing in a closed changing area. All water leaving the test area via drains or trenches was UV-sterilized before release into the RBEBL effluent-treatment system. As mentioned above, all tanks were fitted with plexiglas lids to prevent cross-contamination due to splatter or airborne exchange. Individual siphons fitted to each tank were used to clean the tanks and collect water chemistry samples. The beakers used for water chemistry testing were never allowed to touch the exposure aquaria. Nets and tongs that were used to remove fish and debris from tanks were sterilized with a Betadine solution (200 mg/L) immediately after contact with tanks exposed to bacteria. Temperature, pH, conductivity and D.O. were measured directly in each tank. To prevent cross-contamination, unexposed aquaria were measured first, followed by exposed aquaria. After all of the aquaria were monitored, the probes were sterilized in 70% EtOH for 10 minutes.

Bacterial challenge

The bacterial cultures used in the bacterial-challenge component of this study were prepared by Dr. Rich Holt (Oregon Department of Fish and Wildlife), who traveled to RBEBL to supervise and help conduct this portion of the experiment.

F. columnaris used in the challenges represented a composite of three isolates from the skin and gill lesions of three dead suckers (two Lost River suckers and one shortnose sucker) that Dr. Holt collected during a massive die-off in Upper Klamath Lake in 1996. He cultured the original isolates on cytophaga agar and lyophilized them. Then he combined isolates from these primary cultures with F1 isolates that had passed through juvenile Lost River suckers in a preliminary study conducted to confirm strain virulence.

The *F. columnaris* cultures we used were grown in cytophaga broth at RBEBL in an incubator at ~22 C. They were adjusted by Dr. Holt to an optical density (absorbance) of 0.1 at 525 nm (as measured in a 1-cm pathlength with a Spectronic 20 spectrophotometer) and diluted 1:20 for use in the bacterial challenges. Based on plate counts in cytophaga and TYES agar, colony-forming units in the 100 mL of diluted broth used in all initial challenges ranged from 4.7×10^6 to 5.3×10^6 colony-forming units.

Challenges with *F. columnaris* were performed on Day 30, after fish in the 24 aquaria had been exposed to the various ammonia concentrations for the initial 30-day period. The exposures were performed by first stopping all water flow to the aquaria, then concurrently draining 6.1 L from the 12 control aquaria. These drawdowns were completed in 13 minutes. Then the aquaria designated to receive the bacterial challenge were drained by the same volume and procedures over the following 10 minutes. Immediately after being drained down to 1.9 L, each aquarium received either 100 ml of prepared dilution medium containing *F. columnaris* or 100 ml of sterile medium. To disperse the bacteria in the water, we stirred each aquarium in three slow figure-eight patterns with a small net. After 10 minutes of exposure to the bacteria under static conditions, we resumed water flow to each aquarium.

Monitoring and procedures for handling dead or moribund fish

Three traditional endpoints (survival, length, and weight) were monitored during the experiment. Additional endpoints to assess fish health and pathogen infection were used

following the bacterial challenges in the 12 infected aquaria and 12 matching, uninfected aquaria. Monitoring for dead fish occurred four times daily prior to the bacterial challenge. After the bacterial challenge, all fish were monitored hourly for the first three days, and hourly from 8:00 AM to 8:00 PM thereafter. Endpoints were determined as follows.

Growth and Mortality – Total length of each fish was first measured on a small fish board to the nearest millimeter. Fish were first gently blotted dry, placed in a tared weighing boat and weighed to the nearest 0.01 g. Moribund and dead fish were weighed and measured immediately after retrieving them from the water. Buffered MS-222 was used as the anaesthetizing agent prior to measurements of live fish.

Gross External Anomalies – Gill, skin, eye, skeletal, and fin anomalies were assessed using a ranking system (1-4) of the severity of the anomaly, following the methods of Goede (1989) and Goede and Barton (1990) when possible. Gills were only examined in moribund (rather than dead) fish because of rapid tissue deterioration following death, and gills sampled for *F. columnaris* with a sterile bacteria-transfer loop were not examined to prevent unintentional contamination and/or misinterpretation of damage sources.

Histopathology – Although we planned to preserve moribund fish for histopathology examinations, we were able to retrieve only 3 moribund suckers (i.e., before they died) during the 32-d post-challenge period. Therefore, at the end of the experiment, we randomly selected 6 fish from each aquarium for examination of gills, kidney, and liver at a later date. After measuring and weighing each fish, we anaesthetized it in buffered MS-222 and processed it individually to minimize the amount of time between death and fixation of the tissues. The fish were fixed in Davidson's solution. Prior to this, we removed their opercular flaps to enhance infiltration of the fixative. Additionally, we made abdominal incisions in some of the fish removed from the first several aquaria to also enhance infiltration; we discontinued this in fish from the later aquaria because of the potential for damaging internal organs. After two days in Davidson's solution, we transferred the fish to 70% EtOH. On 4 January 2000, we shipped the fish to Dr. J. Scott Foott (U.S. Fish and Wildlife Service, Fish Health Center, Anderson, California) for examination. All tissue samples were refrigerated from the time they were placed in EtOH until they were shipped to Dr. Foott.

Hematocrit and Red Blood Cell:Leukocyte/Thrombocyte Ratio – We attempted to secure blood for determination of hematocrit and red blood cell:leukocyte/thrombocyte ratios, indicating immune function at the various ammonia concentrations following bacterial challenges. No hematocrit samples were collected from any of the fish because they were too small for adequate blood volume. At the end of the test, two fish from each tank were sampled for red blood cell:leukocyte/thrombocyte ratio. Blood smears were taken from each fish after removing the tails at the caudal peduncle with a razor blade. All blood smears were refrigerated until we shipped them to Dr. Foott on 4 January 2000.

Bacterial Infection – Skin, gills, and kidney of selected fish from each aquarium were cultured to assess rates of *F. columnaris* infection. Because almost all of the fish collected during the exposure were dead, bacterial cultures were only taken from the gills and kidneys. Cultures were taken using a flame-sterilized bacteria-transfer loop. All of the dead and

euthanized fish were cut open using sterile scissors to obtain kidney cultures, and gill cultures were taken after lifting the operculum with sterile forceps. At the end of the test, ten fish from each tank were euthanized in buffered MS-222, wrapped in paper towels, and placed on ice in a styrofoam cooler. These samples were shipped to Dr. Holt on 2 December 1999.

Data analysis

We calculated percentage survival for two time periods -- 1) the first 30 days, and 2) the last 32 days of the experiment. All survival proportions were transformed with the arcsine-square root function (Sokal and Rohlf 1981) before statistical analyses. We compared survival in all seven treatments against survival in the control (the non-challenged, zero-ammonia exposure) using two parametric tests -- Dunnet's test and Williams' test (Lewis et al. 1994, WEST and Gulley 1994). When the concentration response is monotonic, Williams' test is more powerful than Dunnett's test (Gelber et al. 1985). However, the normality assumption was violated for the untransformed and the transformed survival data (Shapiro-Wilk's test); and the treatments with 100% survival had zero variance, compared to significantly non-zero variances in the other treatments. Therefore, we also analyzed survival using two nonparametric tests -- Wilcoxon's Rank Sum test and the Kruskal-Wallis test (Lewis et al. 1994, WEST and Gulley 1994). Although the nonparametric Steel's Many-one Rank test would have been preferable for this study design (Lewis et al. 1994, WEST and Gulley 1994), it could not be conducted because the degrees of freedom in our study did not match those tabulated for that statistical test.

Because they did not violate the assumptions of normality (chi-square test and Shapiro-Wilk's test; WEST and Gulley 1994) and homogeneity of variances (Hartley's test, Bartlett's test, Cochran's test, and Levene's test; WEST and Gulley 1994), transformations of the lengths and weights were not necessary. However, because average length and weight of fish in some aquaria tended to be lower than those in the control aquaria on day 27 of the experiment, we calculated the following ratios for comparisons of growth during the post-bacterial-challenge period (days 31-62): 1) length at day 62:length at day 27, and 2) weight at day 62:weight at day 27. Then we compared those length and weight ratios in all seven treatments against the control using Dunnett's test and Williams' test.

All statistical calculations were performed at $\alpha = 0.05$ using TOXSTAT® Version 3.4 (WEST and Gully 1994).

RESULTS

Summary water chemistry for the 61-d experiment is presented in Table 1. Survival and growth of Lost River suckers during the exposures are presented in Tables 2 and 3, respectively. Raw data for the water chemistry and the biological observations are contained in the EXCEL97 files on the diskette that accompanies this report.

Average water-quality conditions during the 62-day experiment were: temperature - 23.2 C, pH - 9.4, hardness - 48 mg/L as $CaCO_3$, alkalinity - 66 mg/L as $CaCO_3$, conductivity - 165 µS/cm, and D.O. - 7.7 mg/L (Table 1). However, temperature differed slightly for the day 0-35 period (22.3 C) and the day 36-62 period (24.5 C) because we increased the water temperature on day 36. Average unionized ammonia concentrations were 0.006, 0.117, 0.220 and 0.433 mg NH_3-N/L (target concentrations of 0, 0.125, 0.25 and 0.5 mg NH_3-N/L); the corresponding average total ammonia concentrations were 0.010, 0.201, 0.384 and 0.784 mg N/L. On day 26

(i.e., 4 days before the bacterial challenge), the pH controller failed and the pH in all aquaria decreased to 7.0, thus causing all unionized ammonia concentrations to decrease to <0.004 mg NH_3-N/L. However, the pH controller was repaired that day, and the unionized ammonia concentrations were normal the next day (day 27). Variation in the daily unionized ammonia concentrations is shown in Figure 1b.

During the pre-challenge period (days 0-30), ≥96% of the fish survived in the control and in the three sublethal ammonia concentrations (Table 2). After the bacterial challenge (days 31-62), 100% of the fish that were not exposed to *F. columnaris* survived in all four ammonia concentrations; additionally, 100% of the fish that were exposed to *F. columnaris* in the highest unionized ammonia concentration (0.433 mg NH_3-N/L) survived. However, the fish exposed to *F. columnaris* in the lower unionized ammonia concentrations suffered increasingly higher mortality as the ammonia concentration decreased.

Of the 20 fish that died during the post-challenge period, 12 died while the temperature was averaged 22.3 C; the remaining 8 died after the temperature was increased to an average of 24.5 C. All of the deaths that occurred after the temperature increase were in bacterially challenged 0.006, 0.117 and 0.220 mg NH_3-N/L aquaria (3, 2 and 3 deaths, respectively; Fig. 1a).

Using a two-tailed Dunnett's test, survival of bacterially challenged fish in the 0.006 mg NH_3-N/L exposure (85%) was significantly lower than survival in the non-challenged control fish (100%), when all seven treatments were compared to that control. Williams' test produced the same result. When only the 0.117, 0.220 and 0.433 mg NH_3-N/L bacterially challenged treatments were compared to the 0.006 mg NH_3-N/L bacterially challenged treatment, Williams' test also indicated that survival in the two highest ammonia concentrations (0.220 and 0.433 mg NH_3-N/L) differed significantly from survival in the bacterially challenged 0.006 mg NH_3-N/L treatment (95% and 100% survival vs. 85% survival). However, the normality and homogeneity-of-variances assumptions were violated for these parametric tests, even when the data were transformed with the arcsine-square root function. Using non-parametric statistics, neither Wilcoxon's Rank Sum test nor the Kruskal-Wallis test identified significant differences between any of the treatments and the control.

Average initial length and weight (±s.d.) of the juvenile suckers were 47.2 ± 2.40 mm and 0.69 ± 0.118 g (n = 26 fish). Overall, fish size increased by an average of 10.5 ± 3.6 % in length and 39.4 ± 17.6% in weight (n = 24 aquaria). None of the post-bacterial-challenge length or weight ratios in the seven treatments differed significantly from the control (Dunnet's test and Williams' test). Additionally, when only the 0.117, 0.220 and 0.433 mg NH_3-N/L bacterially challenged treatments were compared to the 0.006 mg NH_3-N/L bacterially challenged treatment, none of the post-bacterial-challenge length or weight ratios differed significantly from the 0.006 mg NH_3-N/L treatment (Dunnet's test and Williams' test).

Because bacterial cultures and histopathology were examined by Drs. Holt and Foott, those results are not presented in this report.

DISCUSSION

As we anticipated, exposure to the pathogen *F. columnaris* decreased the survival of juvenile Lost River suckers in some of the bacterial-challenge treatments. However, contrary to our expectations, exposure to sublethal ammonia concentrations at pH 9.5 did not increase the mortality rate. Instead, survival increased as unionized ammonia concentration increased.

The highest unionized ammonia concentration that we tested in the current study (0.433 mg NH_3-N/L) was greater than the highest sublethal concentration (0.37 mg NH_3-N/L) and less than the lowest significant partial-mortality concentration (0.69 mg NH_3-N/L) determined for larval Lost River suckers exposed to ammonia at pH 9.5 in a previous study (Meyer et al. 2000). Growth, whole-body ion content of Ca, K, Na, and Cl, and swimming performance also were not significantly affected at 0.37 mg NH_3-N/L in that study. However, Meyer et al. (2000) reported structural damage to the gills of the larval Lost River suckers at sublethal concentrations as low as 0.20 mg NH_3-N/L, including significantly increased O_2 diffusion distance that was caused by (1) epithelial cell hypertrophy (swelling) and hyperplasia (increased number of cells), (2) mucous cell hypertrophy, and (3) swelling of the lymphatic space between the double epithelial cell layers lining the vascular spaces in the secondary lamellae. Lymphatic swelling was often accompanied by infiltration of white blood cells into the lymphatic space, perhaps indicating stimulation of the immune system or the nonspecific defense system against infections.

The tissue damage and the suckers' compensatory responses at sublethal ammonia concentrations could have influenced their susceptibility to bacterial infection. For example, Smart (1976) reported increased mucus production by gills of rainbow trout exposed to elevated ammonia concentrations. That result suggests Lost River suckers exposed to sublethal ammonia concentrations in the current study might have secreted more mucus than did the suckers not exposed to ammonia. Mucus is thought to help fish defend against infections by trapping bacteria in a layer of mucus that is continually sloughed from the skin and gills (Bond 1996). Therefore, suckers exposed to sublethal ammonia concentrations might have been more resistant to bacterial infection because of their secretion of mucus in response to the concurrent chemical stressor we imposed on them. Additionally, the infiltration of white blood cells into the lymphatic spaces of similarly exposed larval Lost River suckers (Meyer et al. 2000) indicates that the immune system or the nonspecific defense system might have been stimulated in the juvenile suckers in the current study. If so, the ammonia-exposed fish would have been better prepared to defend against a bacterial infection than were the fish not exposed to ammonia.

On the other hand, we cannot currently exclude a direct toxic effect of ammonia on *F. columnaris* as a factor contributing to the increased survival of bacterially challenged Lost River suckers in the higher ammonia concentrations. Although the *F. columnaris* were still viable in those ammonia concentrations (personal communication, Dr. Rich Holt, Oregon Department of Fish and Wildlife), the virulence of the bacteria was not tested.

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provided input to the study design. Elaine Snyder-Conn (U.S. Fish and Wildlife Service) helped design the study; and in our Materials and Methods section, we used portions of a draft manuscript of which she is the lead author.

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Table 1. Water chemistry during the bacterial-challenge experiment with juvenile Lost River suckers -- 30 September - 1 December 1999. Means and standard deviations (s.d.) were calculated from daily measurements of water-quality parameters in all exposure aquaria.

Ammonia exposure level (nominal unionized ammonia conc.)	Mean temperature (C)			Mean pH (±s.d.)	Mean hardness (mg/L as $CaCO_3$) (±s.d.)	Mean alkalinity (mg/L as $CaCO_3$) (±s.d.)	Mean conduct-ivity (µS/cm) (±s.d.)	Mean dissolved oxygen conc. (mg/L) (±s.d.)	Ammonia concentration	
	Entire study (±s.d.)	Days 0-35 (±s.d.)	Days 36-62 (±s.d.)						Total ammonia (mg N/L)	Unionized ammonia (mg NH_3-N/L)
0 (<0.01 mg NH_3-N/L) [control]	23.3 (±1.26) n=124	22.3 (±0.65) n=72	24.5 (±0.65) n=52	9.44 (±0.330) n=124	47.9 (±4.37) n=38	66.2 (±7.67) n=36	164 (±20.9) n=124	7.8 (±0.25) n=106	0.010 (±0.011) n=122	0.006 (±0.006) n=122
1 (0.12 mg NH_3-N/L)	23.2 (±1.30) n=124	22.3 (±0.73) n=72	24.5 (±0.69) n=52	9.43 (±0.329) n=124	47.5 (±1.92) n=31	67.0 (±7.54) n=30	164 (±20.9) n=124	7.8 (±0.32) n=106	0.201 (±0.016) n=122	0.117 (±0.021) n=122
2 (0.25 mg NH_3-N/L)	23.2 (±1.31) n=124	22.3 (±0.77) n=73	24.5 (±0.69) n=51	9.41 (±0.327) n=124	47.6 (±2.03) n=37	65.7 (±7.85) n=37	165 (±20.9) n=124	7.7 (±0.28) n=106	0.384 (±0.023) n=122	0.220 (±0.040) n=122
3 (0.5 mg NH_3-N/L)	23.2 (±1.32) n=124	22.3 (±0.79) n=72	24.5 (±0.64) n=52	9.37 (±0.323) n=124	47.3 (±1.55) n=43	64.3 (±7.74) n=42	168 (±21.1) n=124	7.7 (±0.31) n=106	0.784 (±0.036) n=122	0.433 (±0.078) n=122
Combined data	23.2 (±1.30) n=496	22.3 (±0.73) n=289	24.5 (±0.66) n=207	9.41 (±0.328) n=496	47.6 (±2.69) n=149	65.7 (±7.69) n=145	165 (±20.9) n=496	7.8 (±0.29) n=424	varied among treatments	varied among treatments

Table 2. Survival during the bacterial-challenge experiment with juvenile Lost River suckers -- 30 September - 1 December 1999. Means and standard deviations (s.d.) were calculated from daily observations in all exposure aquaria. * = significantly different from control (i.e., the fish exposed to <0.01 mg NH_3-N/L and not exposed to columnaris).

Ammonia exposure level (nominal unionized ammonia conc.)	Exposed to *Flavo-bacterium columnaris*?	Initial number of fish	Number of fish alive on		Survival (%) (±s.d.) [n=3 per treatment]	
			Day 30	Day 62	Days 0-30	Days 31-62
0 (<0.01 mg NH_3-N/L) [control]	No	75	75	75	100 (±0.0)	100 (±0.0)
1 (0.12 mg NH_3-N/L)	No	75	72	72	96.0 (±6.9)	100 (±0.0)
2 (0.25 mg NH_3-N/L)	No	76	75	75	98.7 (±2.3)	100 (±0.0)
3 (0.5 mg NH_3-N/L)	No	75	75	75	100 (±0.0)	100 (±0.0)
0 (<0.01 mg NH_3-N/L)	Yes	75	74	63	98.7 (±2.3)	85.2 * (±8.2)
1 (0.12 mg NH_3-N/L)	Yes	75	75	70	100 (±0.0)	93.3 (±4.6)
2 (0.25 mg NH_3-N/L)	Yes	75	75	71	100 (±0.0)	94.7 (±6.1)
3 (0.5 mg NH_3-N/L)	Yes	75	74	74	98.7 (±2.3)	100 (±0.0)

Table 3. Lengths and weights during the bacterial-challenge experiment with juvenile Lost River suckers -- 30 September - 1 December 1999. Means and standard deviations (s.d.) were calculated from the means of periodical measurements in all exposure aquaria (n = 3 aquaria per treatment).

Ammonia exposure level (nominal unionized ammonia conc.)	Exposed to *Flavo-bacterium columnaris*?	Date							
		Day 0 (30 September)		Day 18 (18 October)		Day 27 (27 October)		Day 62 (1 December)	
		Length[a] (mm) (±s.d.)	Weight[a] (g) (±s.d.)	Length (mm) (±s.d.)	Weight (g) (±s.d.)	Length (mm) (±s.d.)	Weight (g) (±s.d.)	Length (mm) (±s.d.)	Weight (g) (±s.d.)
0 (<0.01 mg NH_3-N/L) [control]	No	47.2	0.689	48.0 (±0.35)	0.700 (±0.021)	48.4 (±0.20)	0.751 (±0.040)	54.0 (±0.68)	1.073 (±0.076)
1 (0.12 mg NH_3-N/L)	No	47.2	0.689	49.3 (±2.40)	0.832 (±0.154)	49.1 (±2.55)	0.784 (±0.132)	53.5 (±0.77)	1.053 (±0.046)
2 (0.25 mg NH_3-N/L)	No	47.2	0.689	48.3 (±0.50)	0.769 (±0.090)	48.7 (±0.46)	0.705 (±0.051)	53.1 (±1.43)	1.021 (±0.108)
3 (0.5 mg NH_3-N/L)	No	47.2	0.689	47.1 (±2.00)	0.659 (±0.105)	48.4 (±2.00)	0.743 (±0.107)	52.3 (±0.74)	0.959 (±0.058)
0 (<0.01 mg NH_3-N/L)	Yes	47.2	0.689	47.9 (±1.90)	0.737 (±0.082)	47.1 (±0.23)	0.683 (±0.059)	51.6 (±1.96)	0.895 (±0.165)
1 (0.12 mg NH_3-N/L)	Yes	47.2	0.689	47.7 (±0.50)	0.743 (±0.036)	47.3 (±0.81)	0.741 (±0.087)	54.0 (±1.40)	1.108 (±0.055)
2 (0.25 mg NH_3-N/L)	Yes	47.2	0.689	49.0 (±0.72)	0.808 (±0.073)	47.9 (±1.29)	0.722 (±0.081)	53.3 (±0.54)	1.029 (±0.042)
3 (0.5 mg NH_3-N/L)	Yes	47.2	0.689	46.7 (±1.42)	0.622 (±0.043)	48.1 (±1.90)	0.767 (±0.056)	53.3 (±1.03)	1.014 (±0.060)

[a] Average size of 26 suckers randomly selected from the stock population at the beginning of the test.

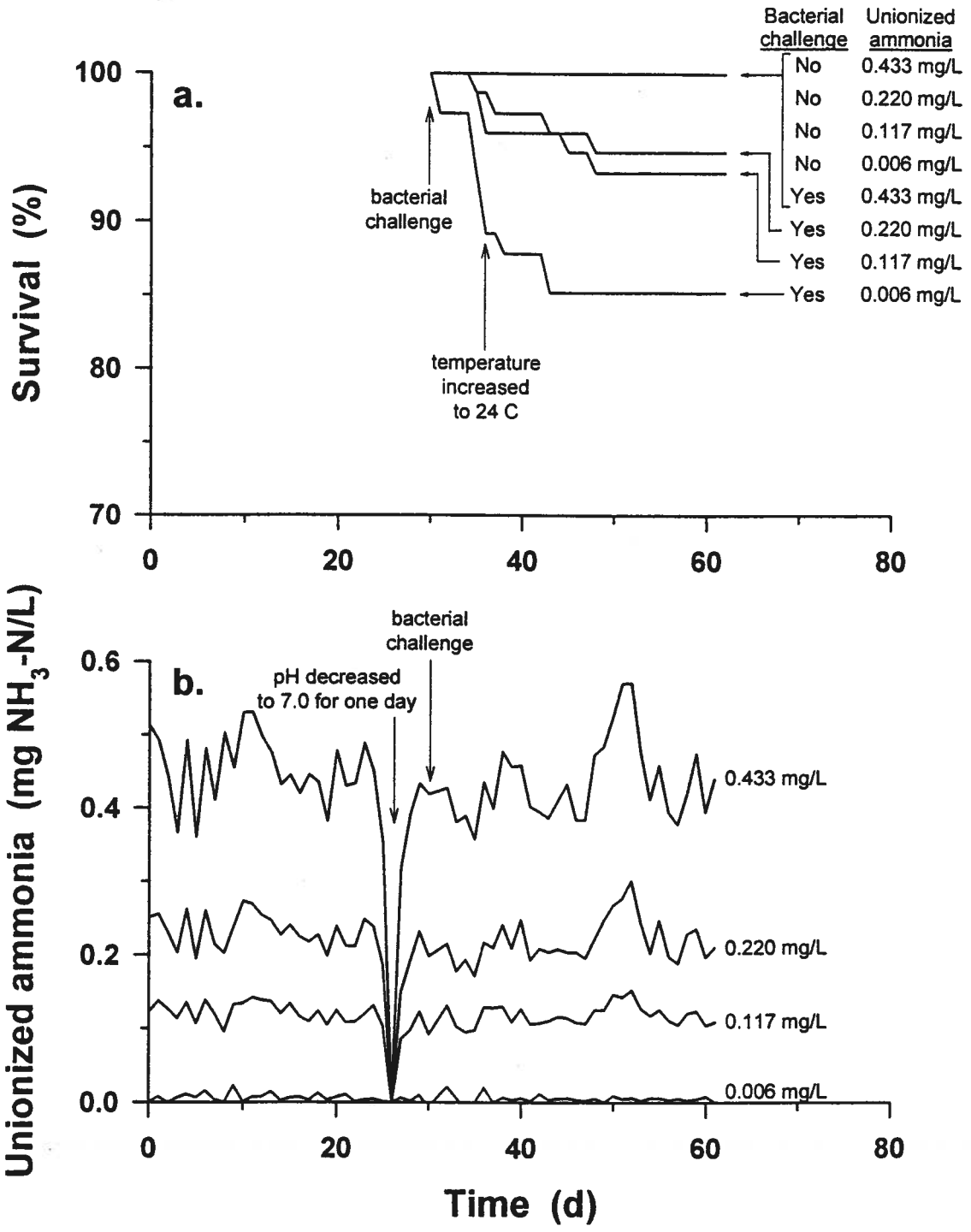

Figure 1. (a) Average survival during the post-challenge period (n = 3 each) and (b) average unionized ammonia concentration (n = 6 each) in the bacterial-challenge experiment with juvenile Lost River suckers -- 30 September - 1 December 1999. Average unionized ammonia concentrations (mg NH_3-N/L) are shown beside the curves. Temperature was 22 C prior to day 36, when temperature was increased to 24 C.